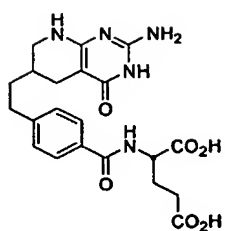
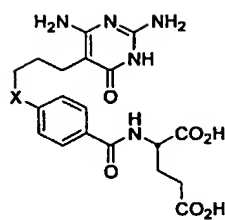


Figure 1

**4, DDATHF****5, X = CH₂, NH, S****Figure 2**

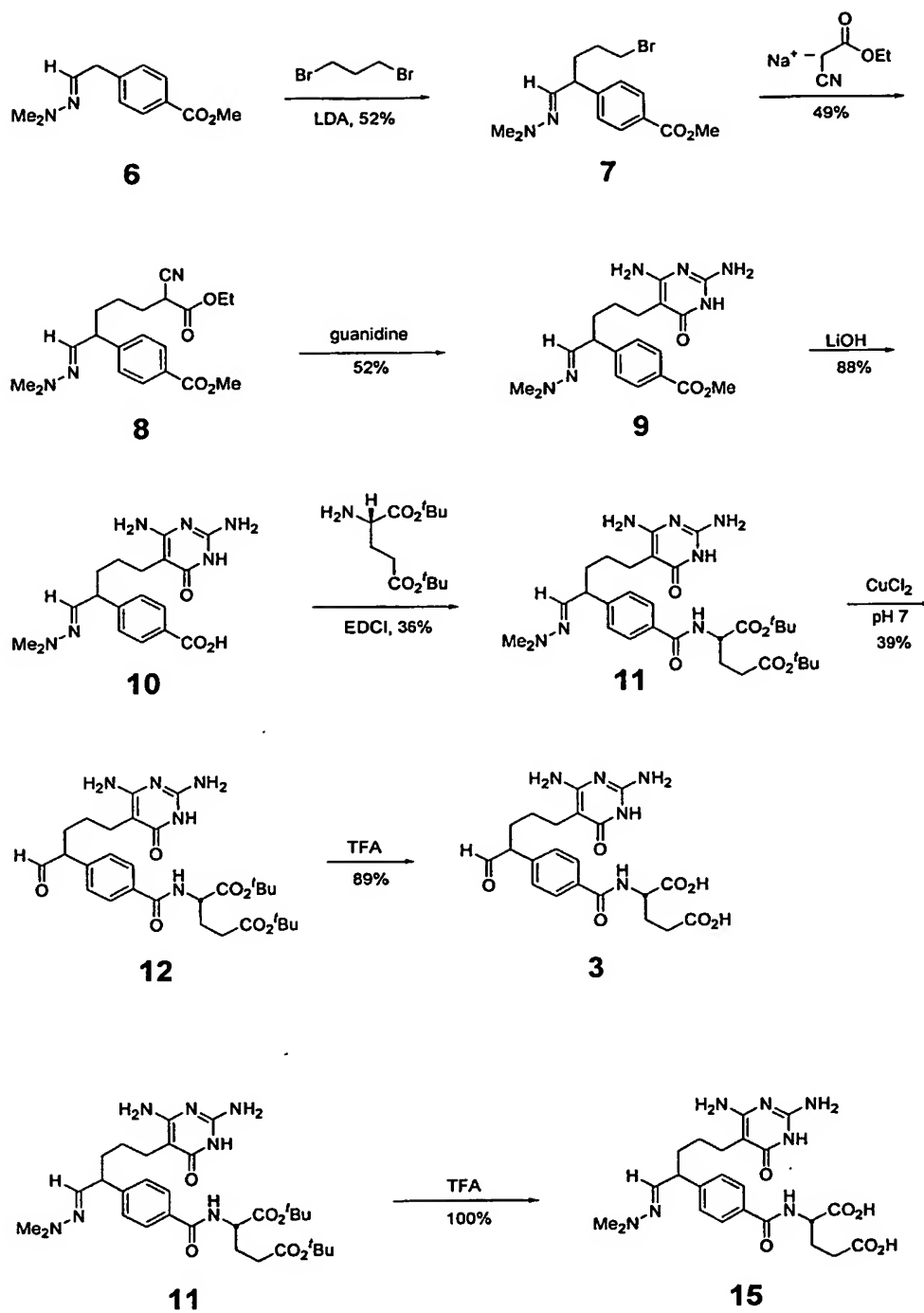
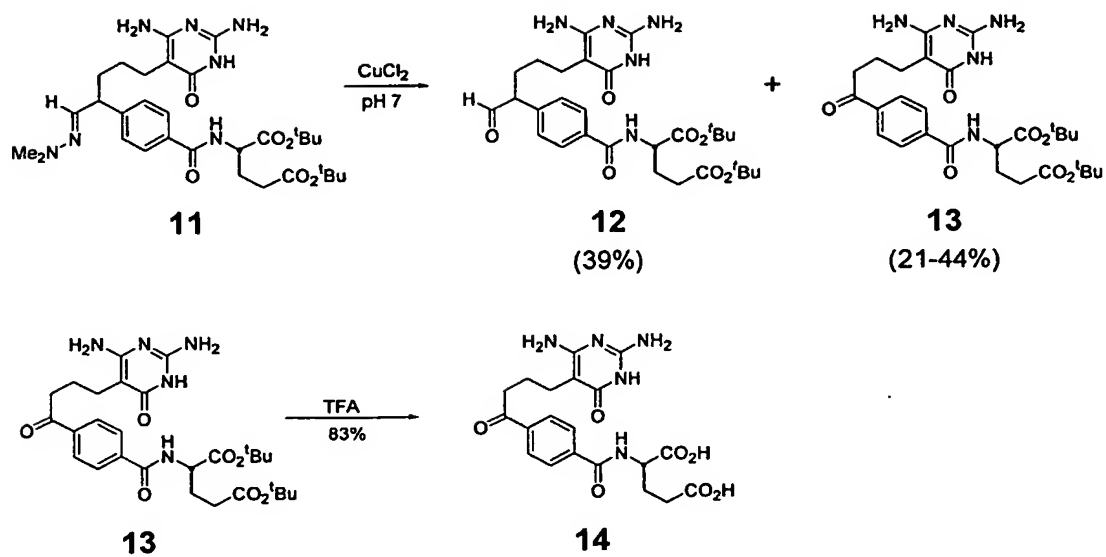
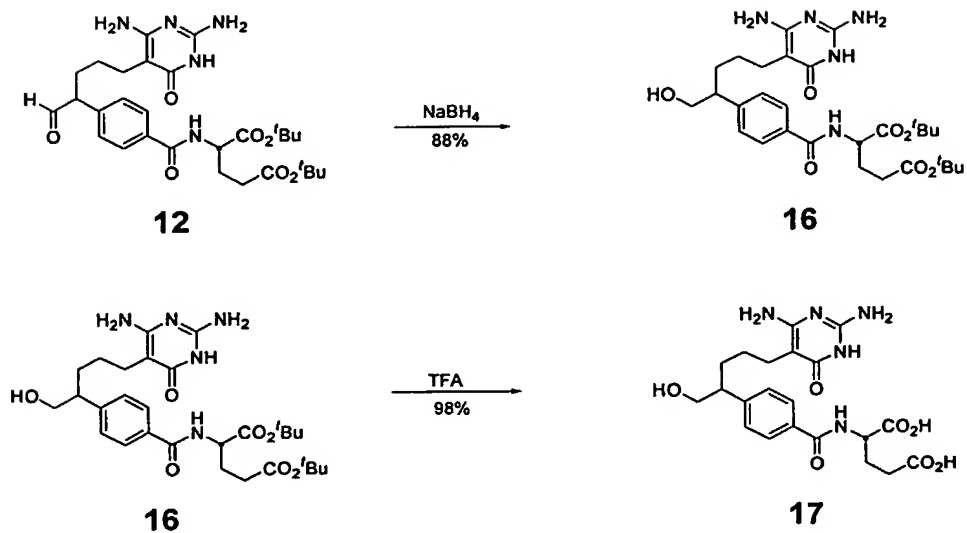


Figure 3

**Figure 4****Figure 5**

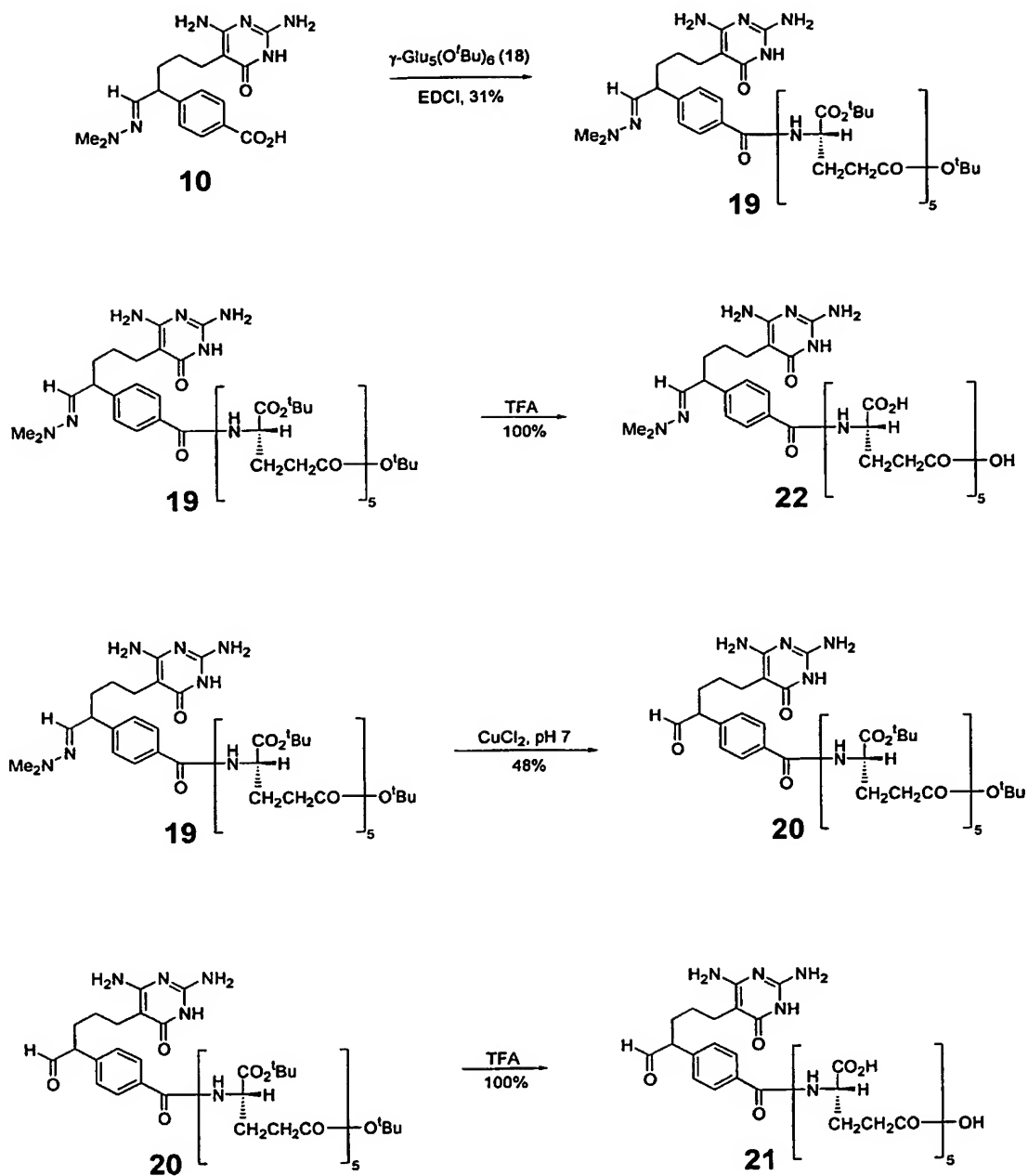


Figure 6

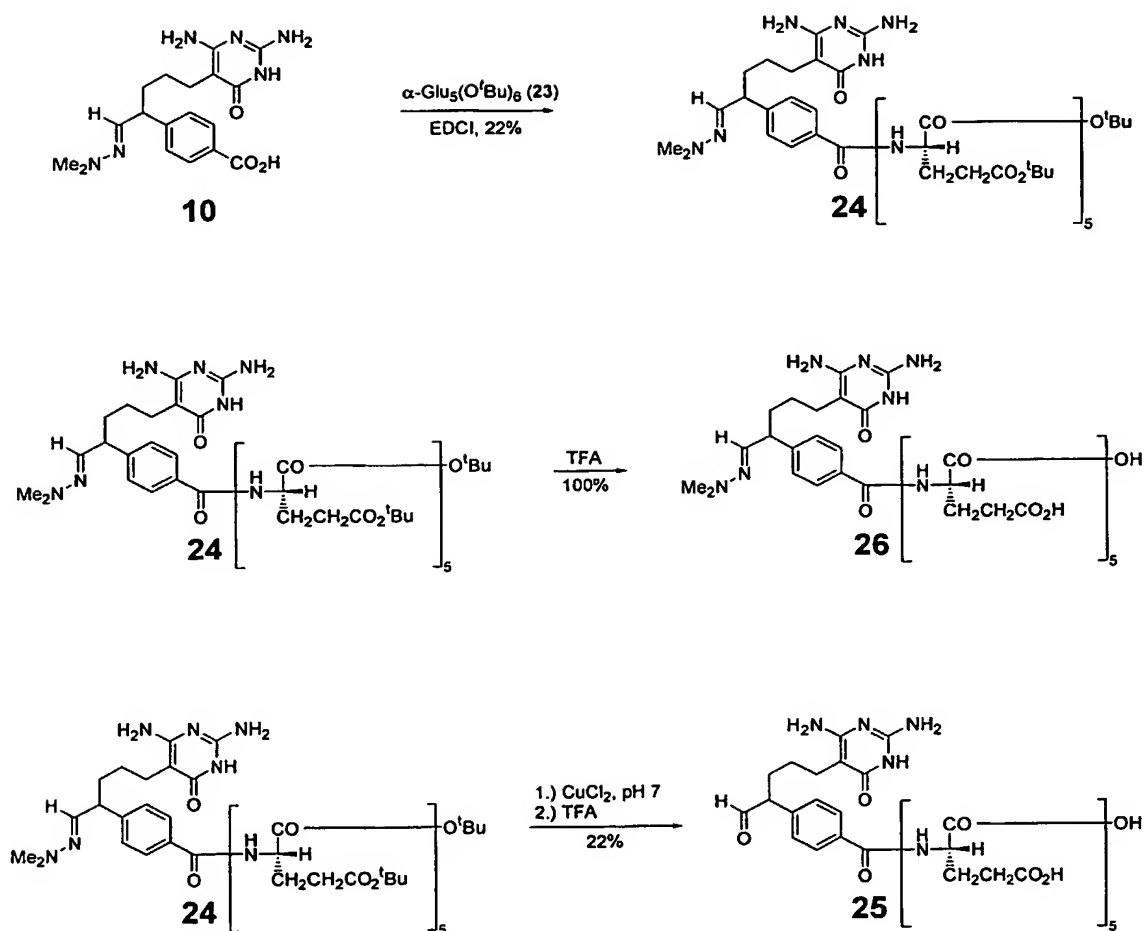


Figure 7

GAR Tfase, AICAR Tfase, and DHFR inhibition (K_i , μM) ^a			
compound	K_i GAR Tfase	K_i AICAR Tfase	K_i DHFR
9	17	>100	>100
10	48	>100	>100
11	>100	>100	>100
12	5	1	>100
3	6	1	>100
14	24	>100	>100
15	6	28	>100
17	16	>100	>100
21	2.7	0.26	25
22	1.9	0.20	62
25	16	16	>200
26	23	7.1	>200
Lometrexol	0.1	nd ^b	nd ^b

^a *E. coli* GAR Tfase, human AICAR Tfase, and *E. coli* DHFR

^bnd, not done

Figure 8

In Vitro Cytotoxic Activity				
compound	CCRF-CEM (IC ₅₀ , μ M)			
	(+) T, (+) H ^a	(-) T, (+) H	(+) T, (-) H	(-) T, (-) H
9	225	>250	80	90
10	>250	>250	>250	>250
11	50	50	50	40
12	50	50	40	50
3	150	170	0.06	0.07
14	80	80	0.20	0.10
15	>200	>200	0.04	0.03
17	>200	160	0.04	0.03
21	>100	>100	>100	>100
22	>100	>100	>100	>100
25	80	60	9	7
26	>100	>100	7	6
Lometrexol	>250	>250	0.20	0.15

^aT = Thymidine, H = Hypoxanthine

Figure 9

In Vitro Cytotoxic Activity in the Presence of AICAR							
CCRF-CEM (IC ₅₀ , μ M)							
compound	(-) T, (-) H, (-) A ^a	(+) T, (-) H, (-) A	(-) T, (+) H, (-) A	(-) T, (-) H, (+) A	(-) T, (-) H, (+) A	(-) T, (-) H, (+) A	(-) T, (-) H, (+) A
3	0.07	0.06	>150	>150	>150	>150	>150
14	0.10	0.20	>200	>200	>200	>200	>200
15	0.03	0.04	>200	>200	>200	>200	>200
17	0.03	0.04	>200	>200	>200	>200	>200
Lometrexol	0.15	0.20	>200	>200	>200	>200	>200

^aT = Thymidine, H = Hypoxanthine, A = AICAR monophosphate

Figure 10

In Vitro Cytotoxic Activity				
CCRF-CEM/MTX (IC ₅₀ , μM)				
compound	(+) T, (+) H ^a	(-) T, (+) H	(+) T, (-) H	(-) T, (-) H
3	130	>200	140	>200
14	>100	nd	nd	>100
15	>200	>200	>200	>200
17	>100	nd	nd	>100
Lometrexol	>200	>200	>200	>200
CCRF-CEM/FPGS ⁻ (IC ₅₀ , μM)				
compound	(+) T, (+) H ^a	(-) T, (+) H	(+) T, (-) H	(-) T, (-) H
3	>100	nd	nd	>100
14	>100	nd	nd	>100
15	>100	nd	nd	>100
17	25	nd	nd	55
Lometrexol	>100	nd	nd	>100

^aT = Thymidine, H = Hypoxanthine

Figure 11

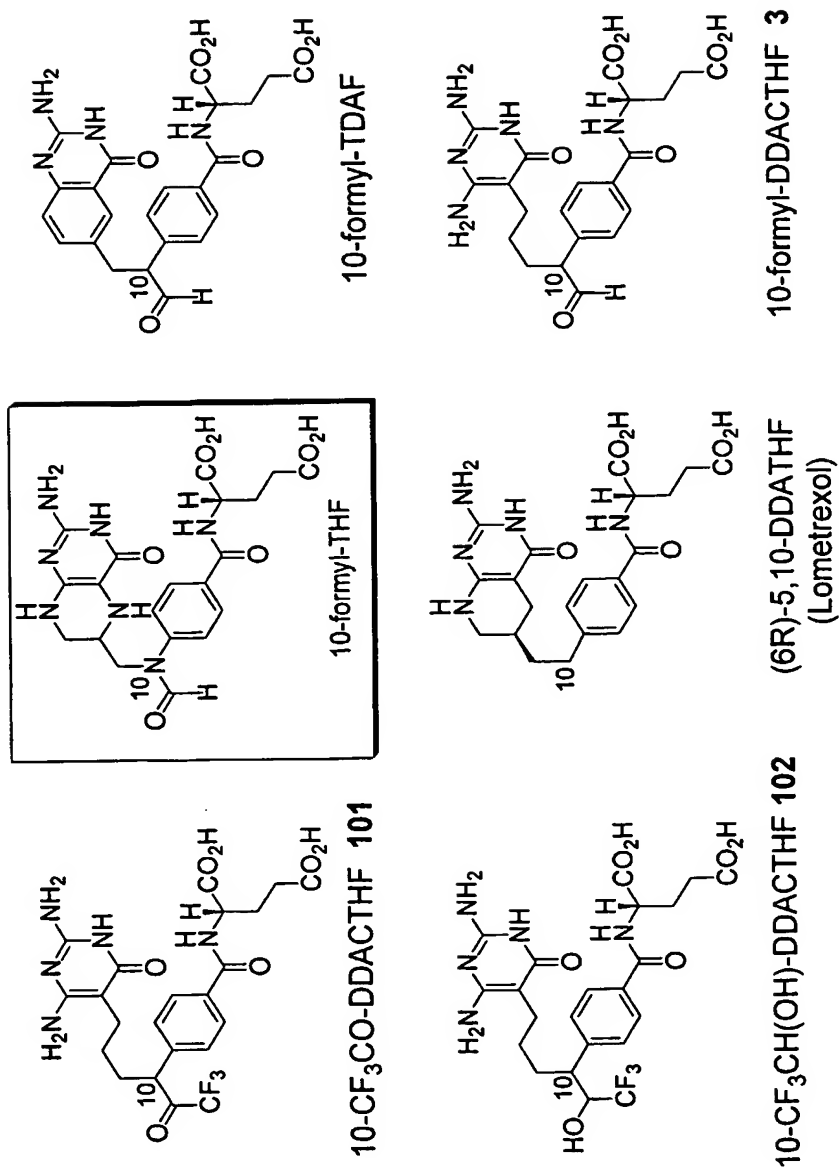


Figure 13

Data Reduction

spacegroup	P3 ₁ 21
unit cell	a = b = 126.24 Å, c = 94.42 Å
no. of molecules per a.u.	2
resolution (Å)	45-1.98 (2.01-1.98) ¹
completeness (%)	99.7 (100)
multiplicity	3.9 (3.8)
average I/σ	24.9 (2.0)
² R _{sym} (%)	7.4 (60.1)

Refinement

data cutoff	F _o > 0σ
reflections (test set)	57912 (2913)
protein atoms	3016
water molecules	251
inhibitor atoms	76
average protein B value (Å ²)	33.1
average inhibitor B value (Å ²)	32.5
average solvent B value (Å ²)	36.8
RMSD from ideal	
bond length (Å)	0.014
bond angle (deg)	1.37
³ R _{cryst} (%)	22.7
⁴ R _{cryst} (%)	24.7
Ramachandran plot (%)	
most favored	92.6
Additionally allowed	7.4

Figure 14



GAR and AICAR Tfase Inhibition (K_i , μ M)			
Compound	<i>E. coli</i> GAR Tfase	rhGAR Tfase	rhAICAR Tfase
10-CF ₃ CO-DDACTHF (101)	1.9	0.015	>100
10-CF ₃ HCOH-DDACTHF (102)	20	0.900	>100
10-formyl-DDACTHF (3)	6	0.14	1
DDACTHF	5	1.7	not determined
Lometrexol	0.1	not determined	not determined

Figure 16

In Vitro Cytotoxic Activity

Compound	CCRF-CEM (IC ₅₀ , μM)			
	(+) T, (+) H	(-) T, (+) H	(+) T, (-) H	(-) T, (-) H
10-CF ₃ CO-DDACTHF (101)	>100	>100	0.017	0.016
10-CF ₃ HCOH-DDACTHF (102)	>100	>100	1.4	1.1
10-formyl-DDACTHF (3)	150	170	0.06	0.07
DDACTHF	>100	>100	3.6	2.7
Lometrexol	>100	>100	0.52	0.23
Methotrexate	0.05	0.05	0.04	0.04

T = Thymidine (+ 10 μM), H = Hypoxanthine (+ 100 μM)

Figure 17

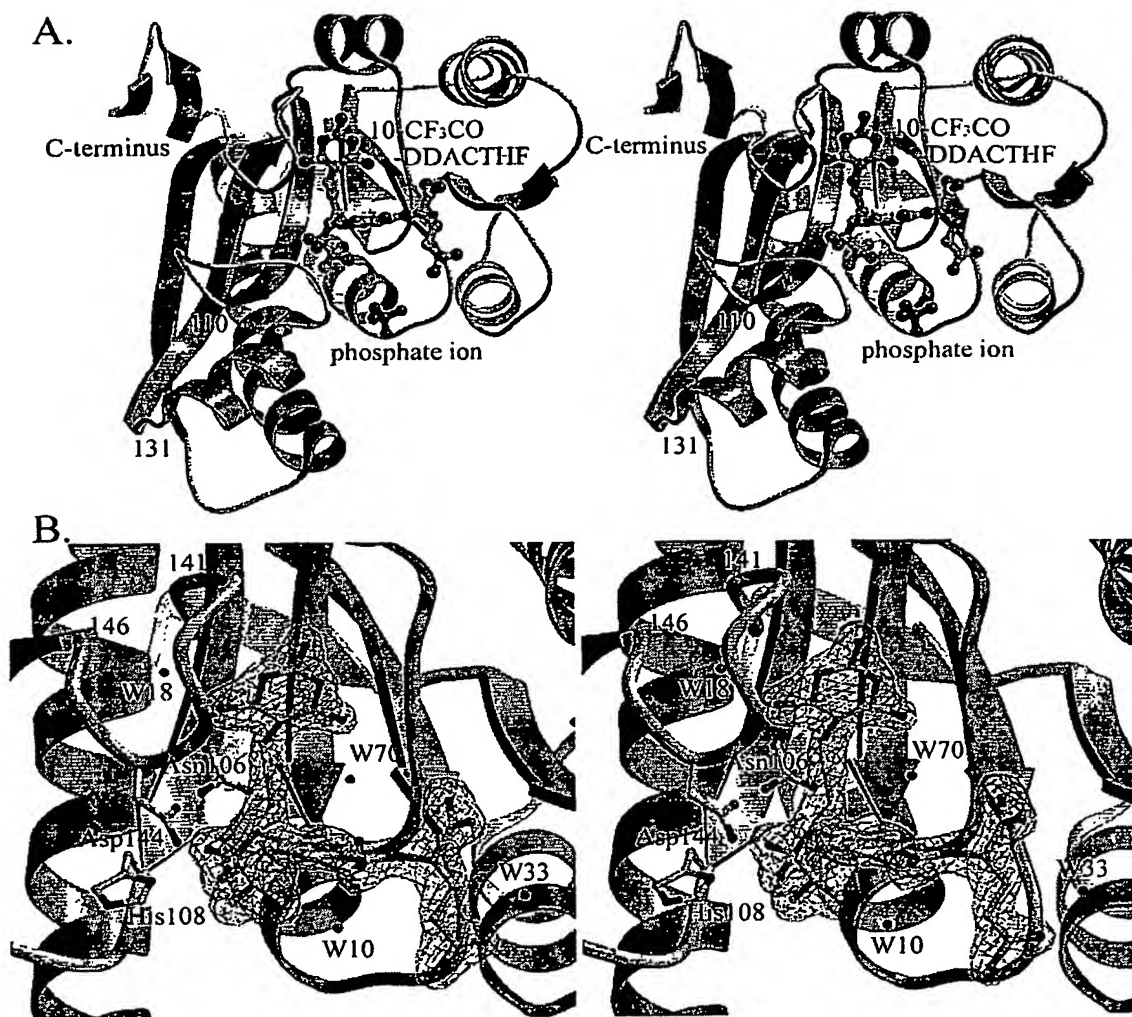
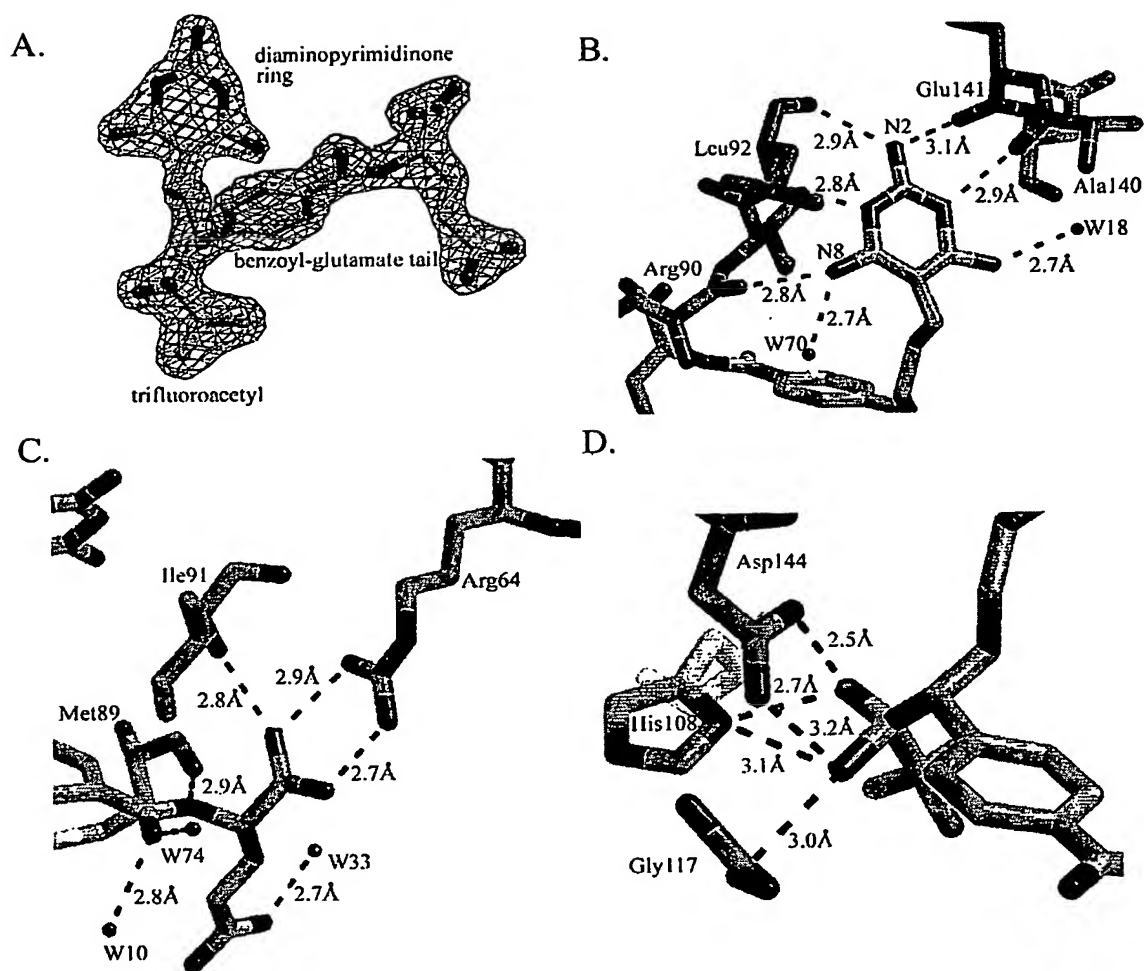


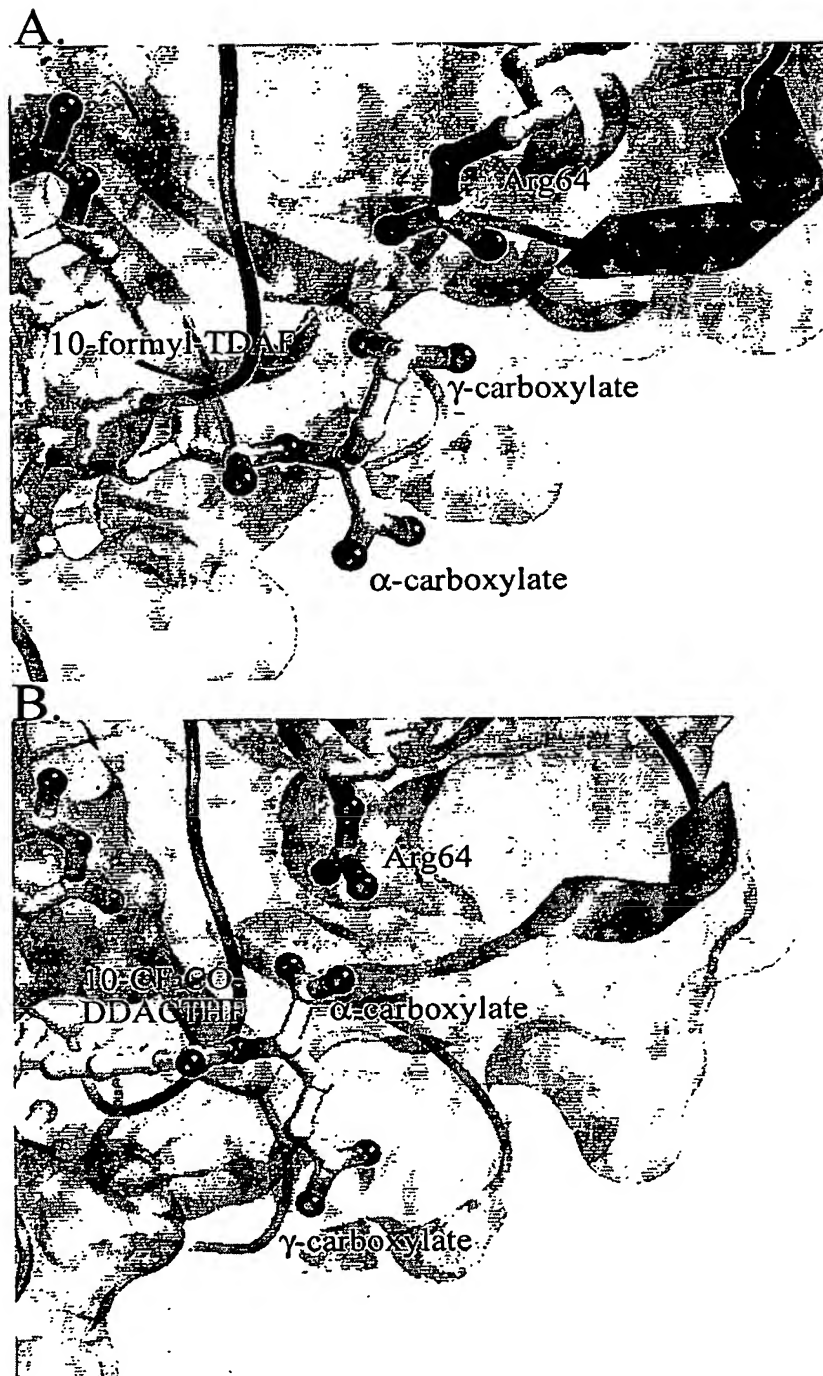
Figure 18

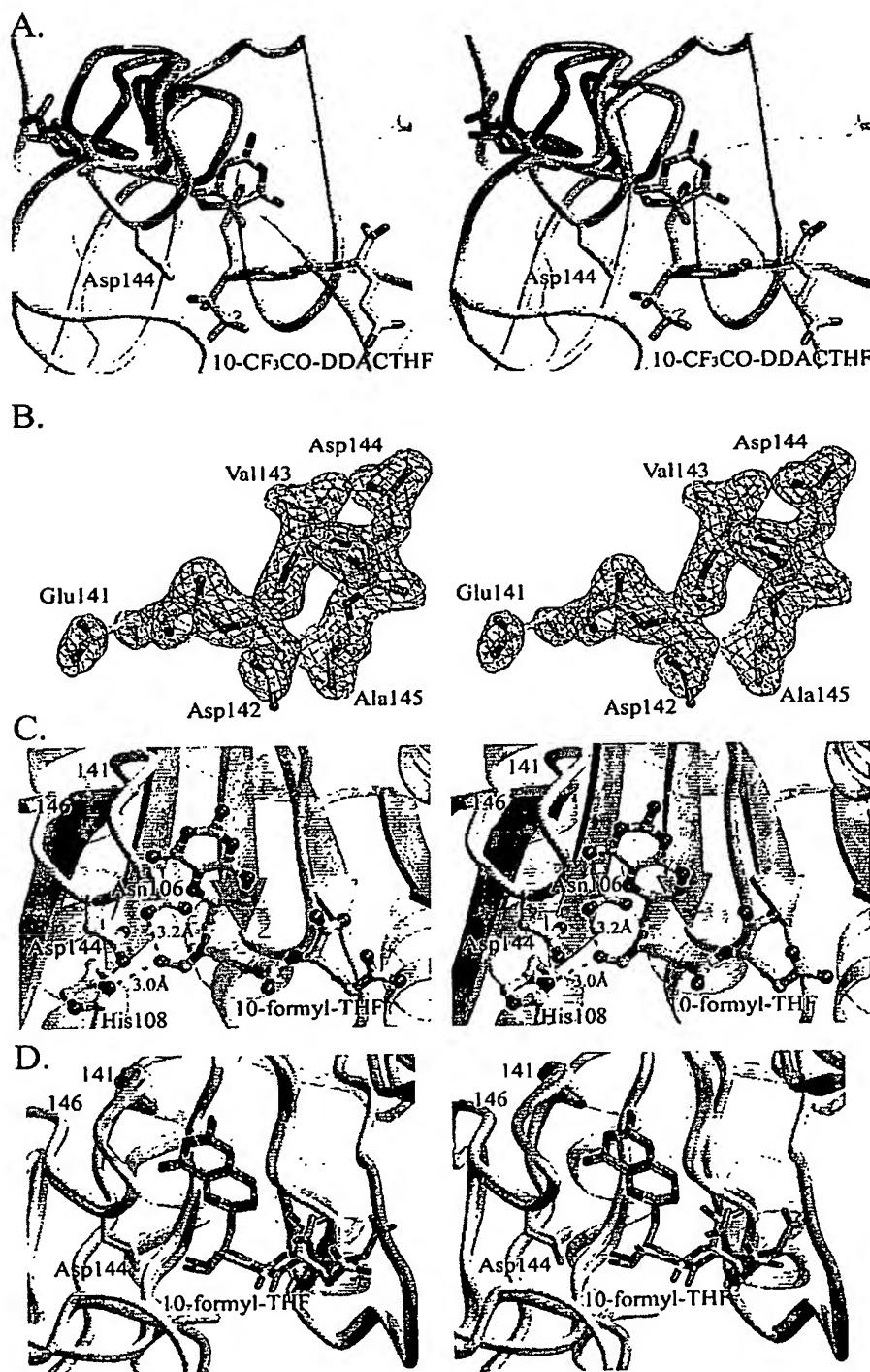
B value comparison of unliganded human GAR Tfase, *E.coli* GAR Tfase in complex with 10-formyl-TDAF and substrate, and human GAR Tfase in complex with 10-CF₃CO-DDACTHF (101)

	Human complex with 10-CF ₃ CO-DDACTHF (101)		<i>E.coli</i> complex with 10-formyl-TDAF-β-GAR	Unliganded human
	Molecule 1	Molecule 2		
	(Å ²)	(Å ²)	(Å ²)	(Å ²)
Protein	31.0	35.3	29.6	30.1
Inhibitor	25.8	39.3	43.5	-
Residues 110-131	22.4	26.6	41.1	23.6
Residues 141-146	30.0	37.5	45.4	64.4

Figure 19

**Figure 20**

**Figure 21**

**Figure 22**

Docking of folate cofactor into human and *E.coli* GAR Tfase structures

Structure of PDB code	Number of clusters	Percentage of conformers in the lowest cluster	Docking E (kcal/mol)	Binding E (kcal/mol)
Human recombinant				
10I	6	49	-19.0	-15.5
apo (1MEJ)	11	15	-16.4	-13.1
<i>E.coli</i>				
10-Formyl-TDAF + β -GAR (1C2T)	2	38	-17.7	-14.5
BW1476U89 (1GAR)	1	100	-16.9	-13.2
Epoxide + β -GAR (1JKX)	3	68	-15.5	-12.2
apo (1CDE)	18	22	-13.9	-11.0

Figure 23